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ON THE LEUCOCYTES OF PEPTONE AND OTHER
VARIETIES OF LIQUID EXTRAVASCULAR
BLOOD.

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“On the Leucocytes of Peptone and other Varieties of Liquid Extravascular Blood.” By A. E. WRIGHT, M.D., Professor of Pathology, Army Medical School, Netley. Communicated by A. D. WALLER, M.D., F.R.S. Received January 30, 1893.

In the course of some investigations on the subject of blood-coagulation, I was led to enumerate the white blood corpuscles in the different varieties of extravascular blood. I propose to report the

results of these enumerations here, and to direct attention to the bearing of the results obtained in the case of peptone blood.

I may premise with respect to the methods employed that the enumerations were made by diluting the blood 200 times with an 8 per cent. solution of magnesium sulphate, which had received a sufficient addition of gentian-violet to stain the cells darkly in the course of a few minutes. The enumerations were invariably made in duplicate samples of each blood, and in each sample the absolute number of leucocytes was counted on 250 squares of the Gowers hæmocytometer. An agreement within 10 per cent. was exacted between the counts of the duplicate samples. When this was obtained the counts were added together, and their sum multiplied by 200 to obtain the total number of leucocytes in the cubic millimetre. The following are the results of my enumerations in oxalated, leech-extract, and peptone blood.

Oxalated Blood.

Obtained by drawing off 9 vols. of blood into 1 vol. of a 1 per cent. oxalate of soda solution.

	Number of white blood corpuscles in normal blood drawn from ear.	Number of white blood corpuscles in blood received from carotid into oxalate solution (figures corrected for dilution effected by the oxalate solution).	Interval between collection of blood into oxalate and commencement of enumeration (<i>i.e.</i> , dilution with MgSO_4 solution).
Dog 1	15,600	15,800	One hour.
Dog 2	10,400	11,200	Half an hour.
Dog 7	20,500	19,700	No interval.
Dog 8	14,000	14,600	Two hours.
Dog 14	22,300	20,600	One hour.
Sum	82,800	81,900	
Average	16,560	16,380	

Leech-extract Blood.

Obtained by extracting 3 vols. of blood from carotid into 2 vols. of leech-extract. The leech-extract was made from leeches which had been kept under alcohol for several weeks.

	Number of white blood corpuscles in normal blood drawn from ear.	Number of white blood corpuscles in blood received from carotid into leech extract (figures corrected for dilution effected by leech-extract).	Interval between collection of blood and commencement of enumeration (dilution with MgSO ₄ solution).
Dog 8	14,000	13,100	Nearly two hours. One hour. Three-quarters of an hour. Quarter of an hour.
Dog 13	16,500	14,250	
Dog 14	22,300	20,000	
Dog 15	12,600	12,500	
Sum	65,400	59,850	
Average	16,350	14,962	

Peptone Blood.

Obtained by injection of 0.3 to 0.5 gram per kilo. of body weight of "peptone" in the form of a filtered 10 per cent. solution in 0.75 per cent. of NaCl.

	Number of white blood corpuscles in normal blood drawn from ear.	Number of white blood corpuscles in peptone blood drawn from carotid.	Interval between peptone injection and blood-letting.	Interval between collection of blood and commencement of enumeration (<i>i.e.</i> , dilution with MgSO ₄).
Dog 1	2,500	10 minutes	One hour.
Dog 6	30,600	3,200	8 "	
Dog 8	14,000	600	5 "	"
Dog 9	19,600	1,800	Not noted	Not noted.
Dog 10	2,800	10 minutes	No interval.
Dog 14	22,300	1,300	2 "	"
Dog 20	14,800	1,600	5 "	"
Dog 21	10,800	600	10 "	"
Dog 22	13,800	800	25 "	"
Dog 23	15,900	400	10 "	"
Sum	8)141,800	10)15,600		
Average	17,725	1,560		
Rabbit 1	7,400	3,600	15 minutes	No interval.
Rabbit 2	10,000	750	3½ hours	
Rabbit 3	8,000	9,200	30 minutes	"
Rabbit 4	8,600	4,600	2 hours	"
		3,300	3 "	"
		4,100	30 minutes	"
		2,500	3 hours	"
		2,800	3½ "	"

The figures in column 2 have not been corrected for the dilution effected by the injection of the peptone solution. Taking the blood as 1/13 of the body weight the amount of peptone solution introduced would effect a dilution of from 3.9 to 6.5 per cent. The figures in column 2 ought therefore to be increased by this fraction in order to be strictly comparable with the figures in column 1.

The above tables show that Dog's peptone blood differs from oxalated, leech-extract, and 8 per cent. $MgSO_4$ blood (this last blood was employed throughout as the standard of comparison) in containing a mere tithe of the normal number of leucocytes. These missing leucocytes have either remained behind in the tissues or in the internal vessels, or they have disintegrated and have passed into solution in the plasma. I have endeavoured to decide between these alternatives by making comparative estimations of the leucocytes in the mesenteric veins, and in the carotid blood, and further by making a series of careful histological examinations of the various organs which might be expected to harbour the leucocytes (I selected the liver, the kidney, and the heart muscle for this purpose). In no case was I able to find any trace of stasis or of emigration of leucocytes either in the Dog or in the Rabbit after peptone injections. I, therefore, feel justified in concluding that in all probability the leucocytes have dissolved in the plasma. I believe that this view is borne out also by a consideration of the chemical properties of peptone plasma, notably by the fact that it deposits on cooling a heavy precipitate of a nucleo-albumen, which is probably identical* with Wooldridge's tissue or cell-fibrinogen, in other words, identical with the characteristic albuminous constituent of the white blood corpuscle.

This "cold precipitate" is not obtained from any other plasma except from oxalate plasma, where I have obtained it, after allowing it to stand for 24 hours before separating it from the white blood corpuscles. Under these circumstances a certain disintegration of white blood corpuscles takes place in this plasma. The non-occurrence† of a "cold precipitate" in leech-extract plasma is in accordance with the fact that this plasma contains no disintegrated leucocytes. The non-occurrence of the precipitate in salted plasmas (Halliburton) probably similarly depends on the fact that the white blood corpuscles do not disintegrate readily in these plasmas, but it may be noted that Wooldridge showed that the addition of neutral salts prevented the precipitation of his "cold precipitate."

We have thus reason to believe that the occurrence of a precipitate on cooling peptone plasma is due to the fact that the plasma contains

* *Vide* Pekelharing's identification of Wooldridge's "cold precipitate," or "A-fibrinogen," with the nucleo-albumen of the cell ('Verhandl. d. Konink. Akad. v. Wetenschappen, Amsterdam,' 2nd Sect., Deel 1, No. 3).

† See Dickinson, 'Journ. of Physiol.,' vol. 11.

leucocytes in solution. If this is so, we have a ready explanation of some of the other characteristics of peptone plasma, notably of the fact that the CO_2 in this blood is remarkably diminished as compared with the normal blood, and also of the fact that peptone plasma clots when a stream of CO_2 is passed through it.

With regard to the diminution of the CO_2 in peptone blood, Lahousse (Du Bois-Reymond's 'Archiv,' 1889) surmised that it was due to a driving out of gas from the blood. He based this view on the extreme rapidity with which this diminution occurred in the blood after peptone injection. Blachstein (Du Bois-Reymond's 'Archiv,' 1891), who followed up Lahousse's work, contributed the following to our knowledge of the question. A diminution of CO_2 is found in the Rabbit's blood as well as in the Dog's blood after the injection of peptone. In the three experiments reported by Blachstein the CO_2 of the normal Rabbit's blood stood to the CO_2 of the peptonised Rabbit's blood approximately in the relation of 4 : 3, 4 : 3, and 3 : 2. In his three experiments on Dog's blood the ratios were approximately 3 : 1, 3 : 1, and 2 : 1. It will be noticed that the CO_2 undergoes a greater diminution in Dog's peptone blood than it does in Rabbit's peptone blood.

Grandis (Du Bois-Reymond's 'Archiv,' 1891) pursued the subject further, and demonstrated that the tension of CO_2 in peptone blood is approximately double that of the CO_2 in normal blood. He indicates that the phenomena point clearly to the liberation of some substance with acid properties in the blood.

In view of these facts I would suggest that this substance with acid properties is in all probability the nucleo-albumen of the white blood corpuscles which have become dissolved in the plasma under the influence of the peptone injection. The liberation of this substance in the blood would result in a driving out of CO_2 from its combination with the bases of the blood plasma, and would thus account for the great diminution of the CO_2 in peptone blood. The differences in this respect between Dog's and Rabbit's peptone blood are in perfect agreement with the results of the enumerations given above for those bloods. The hypothesis of the driving out of CO_2 by a liberation of nucleo-albumen in the blood would further harmonise with the increase of the tension of the CO_2 in peptone blood, and also with the diminished excretion of CO_2 after peptone injections (Bohr, 'Centralblatt f. Physiol.,' 1888).

The fact that casein (better, perhaps, called "caseinogen," Halliburton) will drive out CO_2 from CaCO_3 constitutes an almost perfect analogy with the property of driving out CO_2 which is here surmised to characterise Wooldridge's cell-fibrinogen. In both cases we are dealing with nucleo-albumens.

With respect to the coagulation which is produced in peptone

plasma by passing a stream of CO₂* through it, it appears to me that this might be very naturally explained by assuming that we are dealing with a direct reversal of the process which occurs when peptone is injected into the blood. The CO₂ coagulation in peptone blood would upon this hypothesis be due to a precipitation of cell-fibrinogen in the plasma under the influence of an excess of weak acid. Such a precipitation of cell-fibrinogen in the plasma would be in some sort an equivalent of an addition of cell-fibrinogen to peptone plasma, and would, therefore, naturally inaugurate coagulation.

The coagulation of peptone plasma *in vitro* by CO₂ would be closely paralleled by the fact that intravascular coagulation after injection of cell-fibrinogen occurs, as I have pointed out ('Journ. of Physiol.,' 1890), only in the vascular areas where CO₂ is present in excess.

The precipitation of cell-fibrinogen in peptone plasma under the influence of CO₂ would further have a close analogy in the precipitation of its congener caseinogen from diluted milk by the addition of excess of dilute acids.

* This coagulation does not occur in any other plasma; neither does it occur in blood which has been kept liquid by an addition of peptone *in vitro*. It may be noted that the addition of even 8 per cent. of peptone to blood *in vitro* does not entail any destruction of leucocytes.



